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Organoleptic Evaluation of Three Phenols Present in Wood Smoke

SUMMARY

Guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol, components of wood smoke condensates, were evaluated organoleptically. Mean panel thresholds of the taste and odor in water and the odor in mineral oil were determined for each compound. The majority of the panel characterized the compounds as being smoky in odor and taste, although phenolic characteristics were also present. The relative effectiveness of each compound in the over-all flavor picture was shown by the ratio (concentration in smoke/threshold concentration). Guaiacol had the largest index, whereas 2,6-dimethoxyphenol, which was present in smoke condensate in greatest concentration, had a considerably lower value. Mixing the three compounds in the approximate concentrations found in the smoke condensate did not give the desirable characteristic aroma. Trace components must also be considered for their effect in the over-all flavor pattern.

INTRODUCTION

Food flavors are complex blends of the taste and aroma of the chemical constituents of the products. Modern methods of instrumental analysis have made it possible to separate and identify many flavor components. Although the concentrations of these components may be determined, with a few exceptions these have not been correlated with the effect of the compounds on the sensing apparatus of observers. The relative effectiveness of a compound in the over-all flavor profile is strongly influenced by its limits of sensory detection. Burr (1964) suggested that the concentration of a component in the headspace over a food product, divided by its olfactory threshold, will provide an index of the probable contribution of a compound to total flavor. Thus, many trace components that seem unimportant on a quantitative basis may be key flavor notes, because of their strong odorant qualities.

Meat products exposed to wood smoke acquire a characteristic aroma which is of economic importance. The desirable organoleptic qualities are considered to be due

to the phenolic components in the smoke. The taste and odor thresholds of a phenolic fraction chemically trapped from smoke have been determined (Tilgner *et al.* 1962). These values, however, expressed the total effect of all the components, identity and concentration unknown, in the fraction. Since there are many phenolic compounds in wood smoke, their relative importance in the over-all flavor will be governed by their concentration in the smoke, the concentration required for detection, and their possible interaction with other compounds. Thus, the information needed on the role of the phenolic components in smoke aroma requires analyses dealing with individual phenols. The phenols of a smoke condensate were recently separated in our laboratory, and many were identified (Fiddler *et al.*, 1966). Three phenols—guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol—were present in largest concentration, according to peak areas on the chromatograms. These compounds had been reported previously as products of the dry distillation of wood (Goos, 1952). Guaiacol has been used as the type or class compound for the basic "burnt" aroma in several odor classifications (Crocker and Henderson, 1927; Schutz, 1964), and 4-methyl guaiacol is known as "creosol," a component of wood tar. The present investigation was undertaken to determine the threshold concentrations of these compounds and to evaluate their contribution to the smoky flavor of meat products.

EXPERIMENTAL

Materials. The test compounds—guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol—were obtained from commercial sources. Paraffin oil, N.F., from Fisher Scientific Company was used in experiments requiring mineral oil. Deionized water was prepared initially by passing distilled water through Barnstead Universal Resin. Complaints of off-flavors, however, led to further passage of the water through a charcoal filter. All glassware was washed first with acetone (this step was necessary for removal of the mineral oil but was also

included for aqueous solutions), and then with hot Alconox solution. The glassware was rinsed well with clear, hot water, and finally with deionized water. Distilled water was not used, because it was found to leave an odorous residue in the flasks. The glassware was inverted between rows of wooden rods and allowed to drain dry. Suspending the glassware on the usual draining racks, inverting on towels, and several other methods of drying were tried and discarded because residual odors were noted. All glassware was checked by smelling before use, and odorous pieces discarded.

Gas chromatographic studies were made with a Perkin-Elmer Dual Flame Ionization Chromatograph, model 800, using a single 6-ft \times $\frac{1}{4}$ -inch-OD column packed with 15% Carbowax 20M on 60-80-mesh Gas Chrom P. The temperature was programmed from 70 to 170° at 5°/min. Carrier gas was helium, flowing at 80 ml/min. Injector and detector temperatures were respectively 210 and 200°. Peak areas were determined with a planimeter.

Test panel. The panel consisted of 27 professional members of the staff; approximately 20 members of the panel were available for each test period. Several of the panel had had previous experience with test-panel studies.

Test procedure. Samples of the substance to be tested were freshly weighed each day of testing and immediately diluted with deionized water or mineral oil. Concentrations for testing were obtained by further dilution in glass-stoppered bottles. One-hundred-ml quantities of aqueous solutions and 50 ml of mineral oil solutions were distributed into 250-ml glass-stoppered Erlenmeyer flasks. Several sets of solutions were prepared, each set containing a known water or mineral oil reference blank, a coded hidden blank, and four or five coded concentration levels of the test substance. The solutions were allowed to equilibrate at room temperature for 2 hr before use.

Tests were carried out in the afternoon of the same day each week, and each panelist reported at approximately the same time of day for the tests. For odor tests, panelists were requested to select coded flasks randomly, swirl the contents well, sniff the headspace vapor, and record the absence or presence of odor as compared with the reference blank. With aqueous solutions, the panelist then tasted the solution and indicated the absence or presence of oral stimulus. Retesting was permitted, and no time limit was imposed. Resin- and charcoal-treated water was used for rinsing, and salt-free crackers were available for use between samples to remove after-taste, if necessary.

Calculations. The data were decoded and arranged in order of increasing concentration with appropriate indication of absence or presence of

stimulus. If a taste or odor was indicated in the hidden blank by a panelist, all of his results were eliminated from the computations of that series. Although each determination required a separate, unrelated decision, it was felt that the panelist should be able to identify the hidden blank correctly.

Thresholds of individual panelists for the test substances were determined as the lowest concentration of solution at which taste or aroma was detected. Occasionally a skip occurred, with the panelist recording absence of a stimulus at a concentration higher than his previous positive response. Threshold concentration was then taken as the next-highest concentration above which two or more consecutive positive responses were obtained.

Mean threshold concentrations for the panel were determined by plotting the percent of panelists having thresholds at each concentration level vs. the concentrations of the test solutions and locating the concentration at the 50% response.

Five or six series of analyses were made of each test substance in water or in oil. The first two or three series were considered to be training analyses, to allow panelists to become familiar with the odor and taste of the substances and to determine proper range of concentrations. Training did occur, as shown by the decrease in threshold concentrations reported by the panel. The values of the last three series of determinations, made at constant concentration levels, were used for computation of results. All tests were run at room temperatures ($25 \pm 1^\circ\text{C}$).

RESULTS AND DISCUSSION

Table 1 shows the mean threshold concentrations of guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol for taste and odor in aqueous solution and odor in mineral oil. The lowest concentration of material in aqueous solution detectable by smelling was 0.021 ppm ($1.7 \times 10^{-7} M$) guaiacol. Approximately 5 times as much 4-methyl guaiacol (0.09 ppm), and 90 times as much 2,6-dimethoxyphenol (1.85 ppm), were required for detection by 50% of the panel. Taste thresholds of the aqueous solutions had the same relationship toward each other that was observed for odor thresholds—e.g., the threshold for 2,6-dimethoxyphenol was approximately 100 times that for guaiacol. However, sense of taste appears to be more acute than sense of smell for these compounds, and taste threshold values were somewhat lower. Although stimulation was

Table 1. Mean threshold concentrations of guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol in water and mineral oil.

Phenol	Mean threshold concentration		In oil
	In water	Odor	
Guaiacol	Taste 0.013 ppm ($1 \times 10^{-7} M$)	0.021 ppm ($1.7 \times 10^{-7} M$)	0.07 ppm ($5 \times 10^{-7} M$)
4-Methyl guaiacol	0.065 ppm ($4.7 \times 10^{-7} M$)	0.09 ppm ($6.5 \times 10^{-7} M$)	0.4 ppm ($2.9 \times 10^{-6} M$)
2,6-Dimethoxyphenol	1.65 ppm ($1 \times 10^{-5} M$)	1.85 ppm ($1.2 \times 10^{-5} M$)	0.34 ppm ($2 \times 10^{-6} M$)

applied orally in these experiments, about 90% of the panel reported a loss of taste when they pinched their noses closed during sampling. Thus, the results reported as taste appear to be attributable to stimulation of the olfactory area by passage of vapor through the pharynx and posterior nares. The lower threshold concentrations observed on oral testing may be due to an increase of the vapor pressure of the test substances when raised to body temperature. The number of molecules required to evoke a response should be furnished by a less concentrated solution at body temperature (38°C) than at room temperature (25°C). Increased sensitivity of response to stimulus at higher temperatures has been reported by Baker (1962).

Since meat products contain fat, the effect of interaction of smoke components with this phase must also be considered in the overall flavor picture. Meat fats do not lend themselves as diluents for substances tested in threshold determinations. Vegetable fats such as peanut and soybean oil have characteristic odors that could interfere with flavor determinations. Mineral oil was selected as a bland vehicle for the test compounds, with the reservation that mineral oil, composed of hydrocarbons, may react differently from the fat of meats, with its content of saturated and unsaturated fatty acids. These may undergo chemical reactions with smoke components that modify threshold values. Mean odor thresholds for the test substances in mineral oil are shown in the last column of Table 1. Thresholds for guaiacol and 4-methyl guaiacol were only 3 and 4.5 times as great in mineral oil as in aqueous solution. The threshold concen-

tration of 2,6-dimethoxyphenol, however, was less in oil than in water.

Although many foods contain fat and aqueous phases, little work has been done on the effect of these conditions on flavor. Skramlik (1926) found that taste intensity was greater in aqueous medium because of viscosity effects and the solubility of his test compounds in oil. The thresholds of aliphatic aldehydes decreased in water with increasing chain length to C_{12} (Lea and Swoboda, 1958), while thresholds in oil were not affected through the C_{12} compounds. There was little difference in threshold concentrations with paraffin or peanut oil as solvents. The partition coefficient of flavor components between oil and water may be important in determining flavor thresholds. Patton (1964) found that thresholds of fatty acids decreased with increasing chain length in water, and increased in oil. Volatilization of stimulus is essential for olfactory perception, and bonding to solvent molecules tends to reduce volatilization. Thus, the number of molecules of a compound in the headspace may be greater over a solution in which it is less soluble than over one in which it is more soluble. Guaiacol and 2,6-dimethoxyphenol, which differ in structure by one methoxy group, are slightly soluble in water to the same extent [approximately 1.7 g/100 ml at 16° (Anon.,)]. However, the taste and odor thresholds of 2,6-dimethoxyphenol were about 90 times as great. It is suggested that the volatility of 2,6-dimethoxyphenol is reduced as a result of interaction of the additional methoxy group with the polar solvent. In the nonpolar oil solvent the threshold for 2,6-dimethoxyphenol was of the same order of magnitude

as those for guaiacol and 4-methyl guaiacol. These latter compounds appear to be somewhat more soluble in oil than in water, and, as a result, the concentration required for detection increased.

Threshold concentration is the level at which the presence of a stimulus can be detected. The nature of the stimulus in the present work, however, could not be identified at the threshold level. At somewhat higher concentrations it became possible to characterize the test substance. Initially, the panelists were asked to describe the compounds in their own terms. A dictionary of approximately ten most commonly used adjectives was compiled, and panelists were required to select the most appropriate term(s) from this list. The results of the characterizations are shown in Table 2. The terms "smoky" and "phenolic" were used most commonly, but several additional adjectives of similar nature were grouped with these—e.g., "woody" and "bacon" were counted as "smoky," while "medicinal" and "creosote" were listed as "phenolic." Although approximately 33% of the panelists described the aqueous solutions as having a phenolic taste and aroma, the majority felt the compounds were smoky. The smoky odor was most noticeable for 2,6-dimethoxyphenol, but guaiacol had a more smoky taste. A number of panelists detected a sweet note in the odor and a bitter note in the taste of the three compounds. Characterization of the test compounds was affected by concentration. Many panelists responded differently to high concentrations than they did to lower ones, but a more detailed study of the variations was not made.

The test substances in these experiments were commercial compounds, used without further purification. It has been reported,

however, that impurities may influence the response to a compound (Kendall and Neilson, 1963; Guadagni *et al.*, 1963). The three phenol standards were shown by gas chromatography to contain only traces of unidentifiable contaminants. Triangle tests were used to determine whether there was any difference between commercial and purified materials in odor and taste. The phenols were passed through the gas chromatograph, and sufficient pure material was collected to prepare aqueous solutions containing the mean threshold concentration of each compound. The panel received two test sets for each compound: Set 1 contained two flasks of commercial and one flask of purified preparation; Set 2 consisted of two flasks of purified and a flask of commercial solutions. The panelists were requested to select the odd flask in each set for both odor and taste. There was no significant difference between responses to the commercial and purified preparations of any of the three test compounds. Thus, trace quantities of impurities did not appear to have affected the overall flavor values of test phenols.

Although the effect of any compound in the overall flavor of a complex mixture may depend on a number of chemical and physical parameters, an important consideration is that the concentration be great enough to be detected. The concentrations of the three test compounds were investigated in a representative smoke condensate prepared and fractionated by gas chromatography (Fiddler *et al.*, 1966). The ratio of the areas of the peaks identified as 4-methyl guaiacol, guaiacol and 2,6-dimethoxyphenol was 1:3:4.6. The relationship of peak area to concentration was determined with a standard solution of guaiacol. By calculation,

Table 2. Characterization of the taste and odor of guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol in water.

Compound	% response ^a					
	Odor			Taste		
	Smoky	Phenolic	Sweet	Smoky	Phenolic	Bitter
Guaiacol	53.5	37.5	5.2	58.7	33.5	9.5
4-Methyl guaiacol	51.8	35.5	13.8	43.2	36.3	26.0
2,6-Dimethoxyphenol	71.5	31.5	46.5	34.0	17.0

^a The responses may add up to more than 100% because some judges indicated more than one characteristic for the odor or taste of a phenol.

Table 3. Calculation of flavor index for guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol.

Compound	Conc. in smoke condensate	Flavor index ^a		
		In water		In oil
		Taste	Odor	Odor
4-Methyl guaiacol	$3 \times 10^{-3} M$	6400	4600	1000
Guaiacol	$9 \times 10^{-3} M$	90000	58800	18000
2,6-Dimethoxyphenol	$14 \times 10^{-3} M$	1400	1200	7000

^aConc. in smoke condensate

Mean threshold conc.

the concentration of guaiacol in the smoke condensate was found to be $9 \times 10^{-3} M$. Assuming that the response of the flame-ionization detector was the same for all three compounds, the concentrations of 4-methyl guaiacol, guaiacol, and 2,6-dimethoxyphenol in the smoke condensate were respectively $3 \times 10^{-3} M$, $9 \times 10^{-3} M$, and $14 \times 10^{-3} M$. A flavor index was obtained from the ratio of smoke condensate concentration to mean threshold concentration (Table 3). The most effective compound was guaiacol, in both water and oil. The odor value for 4-methyl guaiacol was somewhat greater than that for 2,6-dimethoxyphenol in water, whereas the latter was more effective in an oil base. An index value of this type is more meaningful than the concentration of a component in a flavor blend, as suggested by Burr (1964).

The flame-ionization gas chromatograph is extremely sensitive, but it has been shown that the nose will often detect odors at lower concentrations than the instrument (Weurman, 1963).

The sensitivity of the nose was greater than the response of the gas chromatograph to the odor of the three smoke components. Aqueous solutions were prepared at odor threshold levels of the three compounds, and 10- μ l quantities were injected into the gas chromatograph. At maximum sensitivity of the detector-recorder combination, no response was observed. To determine the lowest concentration that could be detected by the instrument, several levels of pure guaiacol were injected and the areas of the peaks determined. By calculation, it was established that a minimum detectable response would be obtained from 1.8×10^{-10} moles of guaiacol. The 10 μ l of threshold concentration guaiacol solution (1.7×10^{-7}

M) contained 1.7×10^{-12} moles, or 100-fold less material than that required for minimal response. If headspace vapor over the threshold solution had been used, in closer analogy to the odor-nose relationship, the concentration of guaiacol would be considerably less than 10^{-12} moles. (These computations are limited to the conditions set forth for the gas chromatographic analysis. The flame ionization detector is responsive to the number of carbon atoms present in the sample, so a detectable response could be reached by using a large enough sample).

Although guaiacol, 4-methyl guaiacol, and 2,6-dimethoxyphenol are present in smoke as the most plentiful of the phenols, have threshold levels indicating that they are involved in the total flavor, and have odors described as smoky, mixing the three in the proportions found in smoke condensate yielded a solution only slightly reminiscent of smoke condensate. To evaluate total smoke aroma properly it may be necessary to apply the procedure described herein to all the components in smoke.

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Mention of commercial names does not imply endorsement.